The human granulocytic ehrlichiosis (HGE) agent in Swedish ticks

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Human ehrlichiosis is an emergent tick-borne infection increasingly reported from the USA [1].

Antibodies in humans as evidence of the presence of granulocytic *Ehrlichia* infection have recently been reported from Switzerland [2], the UK [3] and Norway [4] and have been found in Sweden [5]. An *Ehrlichia* strain with identical 16S rDNA nucleotide sequence to that of the agent of human granulocytic ehrlichiosis (HGE), has been identified as causing disease in Swedish dogs and horses [6,7].

Presence of the HGE agent in *Ixodes scapularis* ticks has been demonstrated in the US [8] but not in the related *I. ricinus* ticks of western Europe. We therefore used a PCR-based test, which is specific for the HGE agent, *Ehrlichia equi* and *E. phagocytophila* [7], to study a total of 151 ticks at the nymphal stage, collected on the west and east coasts of Sweden. Of the 151 ticks, 10 (6.6%) were found to contain ehrlichial DNA. Of the 87 ticks from the west coast of Sweden, 8 (9.2%) were positive (Table 1). These ticks had been collected on the Koster islands, where antibodies to the HGE agent had previously been found in serum samples from healthy inhabitants [5]. Of the 64 ticks from the Stockholm area on the east coast of Sweden, two (3.1%) were found to be positive for *Ehrlichia*, and 14.1% for *B. burgdorferi* sensu lato by PCR. No co-infections with *Ehrlichia* and *B. burgdorferi* sensu lato were found.

Table 1 Nucleotide differences close to the 5′ end of the 16S rDNA sequences of *E. equi*, *E. phagocytophila*, HGE agent and Swedish tick ehrlichiae

Ehrlichiae (no. of positive ticks found)	Nucleotide difference at position[a]		
	49	90	98
E. equi	C	A	A
E. phagocytophila	T	A	A
HGE agent	T	A	G
Swedish *Ehrlichia* type I (1)	T	A	G
Swedish *Ehrlichia* type II (3)	T	G	G
Swedish *Ehrlichia* type III (6)[b]	T	G	A

[a]According to the International Union of Biochemistry numbering of the *Escherichia coli* 16S rDNA sequence. GenBank accession numbers: *E. equi* (M73223), *E. phagocytophila* (M73220), HGE agent (U02521).

[b]Including both positives from the east coast.

After extraction of frozen and disintegrated ticks, the DNA was processed by the QIAamp Tissue kit (QIAGEN, Hilden, Germany). The products of the 16S rDNA-based PCR method, approximately 600 base-pairs, were sequenced [7]. The ehrlichiae were found to be of three types (Table 1): one type identical to the HGE agent, three similar to the HGE agent but with a G instead of an A at position 90 and six similar to *E. phagocytophila* but with a G instead of an A at position 90.

Based on 16S rRNA gene sequence analysis of previous studies, all Swedish canine and equine ehrlichiae have been found to be of the HGE type [6,7]. In cattle, *E. phagocytophila* has been demonstrated, but also another *Ehrlichia* strain, which in positions close to the 5′ end of the 16S rDNA differed from both the HGE agent and from *E. phagocytophila*. The nucleotide differences found in the positive ticks in the present study were also close to the 5′ end (Table 1) but in other positions as compared to those of the cattle strain. The importance of these sequence differences remains to be elucidated.

This first demonstration of the presence of ehrlichiae in ticks in a European country, which is endemic for both Lyme borreliosis (LB) and tick-borne encephalitis (TBE), indicates that further studies are needed to identify human clinical cases of ehrlichiosis and of possible co-infections with other tick-borne pathogens. A recent study from the USA [9] has shown both more severe and more protracted LB infection in individuals co-infected with *Babesia microti*. In Europe, *B. divergens* is known to cause human disease and *B. microti* has been isolated from rodents [10]. Co-infections with two or several tick-borne agents, of which *Ehrlichia* is known to be immunosuppressive in animals, may aggravate the clinical picture of LB and TBE also in Europe.

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Multiple intracranial tuberculomas in a non-immunocompromised patient

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Tuberculous involvement of the central nervous system usually appears as tuberculous meningitis or tuberculoma, which often presents as a single intracranial lesion. Multiple intracranial tuberculomas can also be encountered. We report a case of 38 intracranial tuberculomas in a non-immunocompromised patient.

A 33-year-old Laotian man, resident in France for 7 years, was admitted to hospital with a 2-month history of headache, anorexia and sleep disturbances. Treatment with tricyclic antidepressants and benzodiazepines had been instituted 3 weeks before, with no effect. On admission the patient was drowsy, uncooperative and unresponsive. His temperature was 38.6 °C. Examination showed a right Babinski sign; there was no stiff neck, ataxia or visual disturbance. Hematologic and biochemical tests were normal except for an erythrocyte sedimentation rate of 66 mm/h and an alkaline phosphatase level of 173 U/L. Tuberculin test and human immunodeficiency virus serology were negative. Chest X-ray showed diffuse reticulonodular infiltrates.

Contrast-enhanced computerized tomography (CT) scan of the head revealed multiple solid and ring-enhancing lesions, both supra- and infratentorial. At lumbar puncture, the cerebrospinal fluid (CSF) was clear and contained leukocytes at 53/mm^3 (segmented cells 45%, lymphocytes 45%) with a protein level 1.7 g/L, and a glucose level of 0.6 mmol/L (blood glucose: 5.6 mmol/L); no acid-alcohol-fast bacilli (AAFB) were seen.

AAFB were detected in the sputum. A diagnosis of miliary tuberculosis with cerebral tuberculomas was made and a four-drug daily regimen was given with isoniazid 300 mg, rifampicin 600 mg, pyrazinamide 2000 mg and ofloxacin 600 mg associated with prednisolone 60 mg daily. Initial magnetic resonance (MR) imaging demonstrated 20 supratentorial and 18 infratentorial lesions, showing nodular or ring enhancement after gadolinium (Figures 1 and 2). Percutaneous biopsy of the liver demonstrated granulomatous infiltration. After 6 weeks of incubation, sputum cultures grew *Mycobacterium tuberculosis*, which was sensitive to all the first-line antituberculous drugs. Cultures from liver and CSF were negative for *M. tuberculosis*.

After 2 months of treatment, headaches had resolved but changes in mental status persisted. Cerebral MR imaging showed persistence of many lesions. Pyrazinamide and ofloxacin were discontinued. Over the next 6 months, patient had complete resolution of his neurologic signs and miliary shadowing disappeared from the chest X-ray. Prednisolone was gradually discontinued. After 12 months of medical management, follow-up MR examination showed regression of most tuberculomas, and persistence of three periventricular tuberculomas, whose size, however, had clearly decreased.

Diagnosis of intracranial tuberculoma has in the past been confirmed either at surgery or at autopsy [1]. Since the advent of CT, a large number of reported cases have concerned one, sometimes two or three,